

WATER MITE PARASITISM IN FOUR SPECIES OF *Culex* MOSQUITOES AT KANDY, SRI LANKA

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ABSTRACT

The parasitism of *Arrenurus* sp. water mite larvae on adult *Culex tritaeniorhynchus* Giles, *Cx. gelidus* Theobald, *Cx. fuscocephala* Theobald and *Cx. pseudovishnui* Colless was surveyed during October 1986-September 1987 at 4 sites around the city of Kandy in the central province of Sri Lanka. Mosquitoes were collected at weekly intervals using CDC light traps suspended in a piggery at each site. A total of 46,778 female mosquitoes were collected, of each 1,245 (2.66%) were parasitized by 1,803 mite larvae. The number of mite larvae per parasitized mosquito ranged from 1-13 (mean $\pm$ S.D. = 1.45 ± 1.01). Infestation was highest in *Cx. pseudovishnui*, followed by *Cx. tritaeniorhynchus*, *Cx. fuscocephala* and *Cx. gelidus*. Mites were always attached at intersegmental areas, with significantly greater numbers on the abdomen than on the thorax, and least numbers around the head (cervical membrane). Mites on the abdomen attached in significantly greater numbers on the tergal than the sternal surface, but the opposite was seen the thorax. The presence of mite larvae was significantly associated with the nulliparity of the host mosquito.

INTRODUCTION

The Hydrachnidia (water mites) is a group of acarines (subclass Acari; order Acariformes) that has secondarily adapted to an aquatic mode of life (Smith, 1988). Within the 7 super-families in the group, 28 of 45 families and 70 of 300 described genera have representatives in which the larvae form parasitic associations with insect hosts (Smith and Oliver, 1986).

Water mites of the genus *Arrenurus* (Arrenuroidea: Arrenuridae) are common parasites of adult mosquitoes (Diptera: Culicidae), though they also parasitize other insects (Smith and Oliver, 1986). Host seeking larval mites live below the water surface and attach initially to mosquito pupae. They transfer to the adults during emergence and commence feeding on the host haemolymph. About 6 days after attachment to adult mosquitoes, the mites complete feeding and detach from their host. Those larvae that return to water metamorphose into nymphs and later to adults, both of which feed on aquatic

crustaceans. The mites are so specialized for swimming that they cannot leave the water except when attached to a host (Jalil and Mitchell, 1972; Lanciani, 1987). A recent review on water mite parasitism (Smith, 1988) shows aspects of the mite-mosquito relationship that are of interest in relation to the dynamics of disease transmission by the mosquito: there is evidence that mite parasitization may adversely affect the survival, fecundity and flight activity of the host. The presence of mite larvae on the female mosquitoes may serve as markers of host nulliparity and be useful in the age-grading of vector populations.

There have been no previous studies on the ecology of water mites parasitizing mosquitoes in Sri Lanka. In view of the implications of this relationship to the capability of disease transmission by the host, we carried out a preliminary survey of water mite parasitism in 4 species of mosquitoes, *Culex tritaeniorhynchus* Giles, *Cx. gelidus* Theobald, *Cx. fuscocephala* Theobald and *Cx. pseudovishnui* Colless, that have been implicated in the field carriage of Japanese encephalitis virus in Sri Lanka (Peiris *et al.*, 1987; Amerasinghe *et al.*, 1988, 1989). Aspects investigated were mite prevalence, distribution on the host and the relationship with the age status (parity) of the host. The results reported in this paper show that mite prevalence varied significantly between host species and study sites, and between different areas of the host body, while mite occurrence was significantly associated with the nulliparity of the host.

MATERIALS AND METHODS

The study was carried out during October 1986 - September 1987 around the city of Kandy in the Central Province of Sri Lanka (latitude 5°55' - 9°51' N, longitude 79° 41' - 81°53' E), situated in the upland wet zone of the island at a mean elevation of 530 m. above sea level. Mean daily temperature in the area ranged from 13 - 35.9° C and mean annual rainfall from 1900 - 2500 mm.

Mosquitoes were collected from 4 study sites: (i) Pilimalalawa, a low-lying village area dominated by rice fields and home gardens, situated on the western outskirts of the city; (ii) Udaperadeniya, a hilly village area of mainly wooded slopes and some rice fields, also on the western flank of the city; (iii) Ampitiya, a hilly semi-urbanized area with a moderate extent of rice fields, situated on the southeastern flank of the city; (iv) Bahirawakanda, a semi-urbanized area of grassy slopes within the municipal area of the city.

Mosquito collections were done at weekly intervals using two CDC light traps operated between dusk and dawn in a piggery at each site. Trapped mosquitoes were taken live to the laboratory, anaesthetized using chloroform and identified to species. Female *Cx. tritaeniorhynchus*, *Cx. gelidus*, *Cx. fuscocephala* and *Cx. pseudovishnui* parasitized by mite larvae were further examined for the number and distribution (head-neck, thorax and abdomen) of the parasites. The larvae were then detached, mounted temporarily on glass slides and identified under a compound microscope using the keys of Mullen (1974) and the description of Jalil and Mitchell (1972). Randomly selected samples of parasitized and unparasitized *Cx. pseudovishnui* and *Cx. tritaeniorhynchus* were subjected to parity dissections using Detinova's (1962) technique. Parasitological terminology used here follows Margolis *et al.* (1982), where *prevalence* refers to the total number of parasitized hosts divided by the total number of potential hosts and *intensity* refers to the number of parasites on an individual host.

Statistical analysis of the level of parasitization at different sites and different species was by Single Classification Analysis of Variance (ANOVA) followed by the Sum of Square Simultaneous Test Procedure (SS-STP) (Sokal and Rohlf, 1969). Parasite distribution on the host body was examined by Chi Square (χ^2) Goodness-of-Fit tests and parasitism in relation to host parity status by 2 x 2 Contingency χ^2 tests (Sokal and Rohlf, 1969).

RESULTS

46,778 females of the 4 *Culex* species studied were collected. Of these, 1,245 (2.66%) were parasitized by 1,803 mite larvae. Mites did not occur on 129 males (06 *Cx. tritaeniorhynchus*, 05 *Cx. gelidus*, 94 *Cx. fuscocephala*, 24 *Cx. pseudovishnui*) collected during the study. The mites belonged to the genus *Arrenurus*; all were similar in external features and probably belonged to a single species, but further identification was not possible on the characters of the larval stage.

Table 1. Site and species related parasitization of *Culex* mosquitoes by *Arrenurus* mite larvae

	Pilimalalawa	Uda-Peradeniya	Ampitiya	Bahirawakanda	ALL SITES
<i>Cx. tritaeniorhynchus</i>					
NP / NE	146 / 8143	2 / 295	0 / 210	1 / 96	149 / 8744
Prevalence (%)	1.79b	0.68	0	1.04	1.70
<i>Cx. pseudovishnui</i>					
NP / NE	893 / 14360	116 / 3661	11 / 1021	7 / 427	1027 / 19469
Prevalence (%)	6.22a	3.17b	1.08b	1.64b	5.28
<i>Cx. fuscocephala</i>					
NP / NE	47 / 11891	0 / 452	0 / 402	1 / 214	48 / 12959
Prevalence (%)	0.40	0	0	0.47	0.37
<i>Cx. gelidus</i>					
NP / NE	19 / 4439	2 / 913	0 / 166	0 / 88	21 / 5606
Prevalence (%)	0.43	0.22	0	0	0.37
ALL SPECIES					
NP / NE	1105 / 38833	120 / 5321	11 / 1799	9 / 825	1245 / 46778
Prevalence (%)	2.85	2.26	0.61	1.09	2.66

NP = Number Parasitized; NE = Number Examined.

Different letters following Prevalence values down the "Pilimalalawa" column indicate significant ($P < 0.05$) differences in parasitization among mosquito species. Different letters following prevalence values along the "*Cx. pseudovishnui*" row indicate significant ($P < 0.05$) differences in parasitization among sites (Tested by Single-Classification ANOVA and SS-STP procedure).

Table 2. Mean Intensity of parasitization by *Arrenurus* mite larvae

	Pilimalalawa	Uda-Peradeniya	Ampitiya	Bahirawakanda	ALL SITES
<i>Cx. tritaeniorhynchus</i> (n = 149)	1.29 ± 0.75	(1.00)	-	(1.00)	1.28 ± 0.77
<i>Cx. pseudovishnui</i> (n = 1027)	1.53 ± 1.05	1.28 ± 0.58	1.00 ± 0	(1.43)	1.49 ± 0.97
<i>Cx. fuscocephala</i> (n = 48)	1.26 ± 0.83	-	-	(1.00)	1.25 ± 0.82
<i>Cx. gelidus</i> (n = 21)	1.05 ± 0.58	(1.00)	-	-	1.05 ± 0.55
ALL SPECIES (n = 1245)	1.47 ± 1.00	1.28 ± 0.59	1.00 ± 0	(1.11)	1.45 ± 1.01

"a" = mean number of mite larvae ± S.D. per parasitized female mosquito.

"n" = Number of parasitized female mosquitoes.

Figures in parentheses refer to cases where > 10 parasitized mosquitoes were available. No Standard Deviation is provided in these cases.

Mite parasitization of the 4 mosquito species occurred consistently only at Pilimalalawa, and *Cx. pseudovishnui* was the species that showed the highest level of parasitization across all sites (Table 1). Thus species comparisons were based on data from Pilimalalawa and site comparisons on *Cx. pseudovishnui*. ANOVA and SS-STP tests based on the monthly mean percentage parasitization values at Pilimalalawa showed that mite occurrence was significantly higher ($P < 0.05$) in *Cx. pseudovishnui* than in *Cx. tritaeniorhynchus*, and both were significantly higher than that in *Cx. fuscocephala* and *Cx. gelidus*. Similar statistical tests showed that parasitization in *Cx. pseudovishnui* was significantly higher at Pilimalalawa than at other sites, differences among Udaperadeniya, Ampitiya and Bahirawakanda were non-significant.

The number of mite larvae per infested mosquito ranged from 1-13. The intensity of parasitization was fairly even among species and across sites; highest levels, however, were shown in *Cx. pseudovishnui* (Table 2). Statistical testing was not attempted owing to the very small samples of parasitized mosquitoes recorded in 3 of the 4 species investigated (see data in Table 1).

Mites were attached always to membranous intersegmental parts of the mosquito body. Their distribution in *Cx. tritaeniorhynchus* and *Cx. pseudovishnui* at the Pilimalalawa site is shown in Table 3. In both species, mite number increased progressively and significantly from head to abdomen. Significantly more

Table 3. Number (%) of mite larvae infesting different body regions of two *Culex* species at the Pilimalawa

	<u><i>Cx. tritaeniorhynchus</i></u> (n = 146)	<u><i>Cx. pseudovishnui</i></u> (n = 893)
i. Distribution on Body		
Head	9 (4.02)a	6 (0.43)a
Thorax	40 (17.86)b	136 (9.66)b
Abdomen	175 (78.12)c	1265 (89.91)c
Total	<u>224 (100.0)</u>	<u>1407 (100.0)</u>
ii. Distribution on Thorax		
Terga	1 (2.50)a	1 (0.74)a
Sterna/Coxae	37 (92.50)b	134 (98.52)b
Pleura	2 (5.00)a	1 (0.74)a
Total	<u>40 (100.0)</u>	<u>136 (100.0)</u>
iii. Distribution on Abdomen		
Terga	98 (56.00)a	768 (60.71)a
Sterna	61 (34.86)b	423 (33.44)b
Pleura	16 (9.14)c	74 (5.85)c
Total	<u>175 (100.0)</u>	<u>1265 (100.0)</u>

Statistical testing by χ^2 Goodness-of-Fit tests: Dissimilar letters along columns within each module are significantly different ($P < 0.01$).

"n" = Number of parasitized female mosquitoes

mites occurred on the venter of the thorax than on the dorsum or sides, but the opposite was seen on the abdomen. the distribution on the thorax was probably related to the availability of easily pierced intersegmental membranes only on the ventral aspect. Intersegmental membranes ringed the abdomen, allowing easy access to feeding mites; thus the predominantly dorsal distribution of mites on the abdomen can be best explained by the greater protection afforded under the wings on the abdominal tergal surface, compared to the more exposed pleural and sternal surfaces where mites were open to the risk of detachment by abrasion on resting or feeding surfaces.

Parity dissections of parasitized and unparasitized female *Cx. tritaeniorhynchus* and *Cx. pseudovishnui* collected during a population peak in October 1986 and a trough in May 1987 showed that mite occurrence was significantly associated with the nulliparity of the host mosquito (Table 4.). Only 11.8% (11/93) of parasitized females were parous.

Table 4. Parity of randomly dissected mite-parasitized and unparasitized females of two *Culex* species at the Pilimalawa study site.

	<u>Nulliparous</u>	<u>Parous</u>	<u>Total</u>
<u>Cx. tritaeniorhynchus</u> (October 1986)			
No. Parasitized	18	2	20
No. Unparasitized	94	68	162
Total	<u>112</u>	<u>70</u>	<u>182</u>
$(\chi^2 = 7.69, DF = 1, P < 0.01)$			
<u>Cx. pseudovishnui</u> (October 1986)			
No. Parasitized	39	4	43
No. Unparasitized	195	206	401
Total	<u>234</u>	<u>210</u>	<u>444</u>
$(\chi^2 = 28.02, DF = 1, P < 0.001)$			
<u>Cx. pseudovishnui</u> (May 1987)			
No. Parasitized	25	5	30
No. Unparasitized	23	15	38
Total	<u>48</u>	<u>20</u>	<u>68</u>
$(\chi^2 = 4.20, DF = 1, P < 0.05)$			

Statistical testing by 2 x 2 Contingency χ^2 tests.
 DF = degrees of freedom. Note: Too few *Cx. tritaeniorhynchus*
 were collected during May 1987 for analysis.

DISCUSSION

Larvae of *Arrenurus* mites have been reported to parasitize at least 9 genera of mosquitoes world-wide, including *Anopheles*, *Culex*, *Culiseta*, *Coquillettidia*, *Mansonia*, *Aedes*, *Uranotaenia*, *Ficalbia* and *Hodgesia* (Mullen, 1974). The present study is the first on mosquito parasitization by water mites to be reported from Sri Lanka. In addition to the 4 *Culex* species studied here, other frequently parasitized species observed in Sri Lanka include *An. peditaeniatus* (Leicester), *An. vagus* Donitz and *Ma. uniformis* (Theobald) (unpublished data of F. P. A.).

Differences in water mite prevalence among the 4 *Culex* species in the present study could reflect breeding habitat preference by the mosquitoes of host selectivity by the mites. It is interesting that the two mosquito species showing the highest level of parasitization, i.e., *Cx. tritaeniorhynchus* and *Cx.*

pseudovishnui, are closely associated with rice field breeding in Asia (Reuben 1971, Sirivanakarn 1976, Amerasinghe and Ariyasena, 1990). Site-related distribution in the present study supported this: mosquito and mite abundance was highest at Pilimalalawa which was the site where rice cultivation was the most extensive. Host specificity on the part of the mites too may be an important factor: for instance, Jalil and Mitchell (1972) report 2 morphologically similar species of *Arrenurus* to have non-overlapping host preferences on *Mansonia* and *Anopheles* species respectively. Smith (1988) comments that host specificity probably reflects specialized habits or environmental needs of the mite that limit the range of available hosts. Within the usual host range, natural primary hosts are the most preferred or susceptible to parasitization in the laboratory. Differences in the extent of infestation among host species can be attributed to host morphology, defensive behavior or selection by the mite.

The present data showed that mite larvae attached exclusively to female *Culex*. Host gender differences in level of parasitization have been reported in the field but not demonstrated in the laboratory. Apparent sexual preference may be due to male mosquitoes emerging before females, or to behavioural differences resulting in the greater exposure of one sex to larval mites (Smith, 1988). The survival value to a water mite of attaching to a female mosquito is obvious: this host gender can be guaranteed to return to water to oviposit, thereby affording an opportunity for the mite to detach into the habitat for which it is adapted. The distribution of mite larvae on the host mosquito too, may be species-specific. Most larvae have characteristic location of attachment, which depend on the species of mite, but may vary with host species and gender (Smith, 1988). Jalil and Mitchell (1972) reported two *Arrenurus* species to have different attachment patterns: a species parasitizing *Mansonia* occurred most on the thoracic coxae, followed by the cervical region and least on the abdomen; a species on *Anopheles* occurred more on the abdomen than the thoracic coxae and was absent on the cervical area. The Sri Lankan *Arrenurus* of the present study showed a pattern similar to the latter, except that a few larvae attached to the cervical region as well. The distributional pattern was similar in both *Cx. tritaeniorhynchus* and *Cx. pseudovishnui*, an indicator that the same species of mite occurred on both mosquitoes.

Gillett (1957) advanced the view that the presence of parasitic water mite larvae was a possible indicator of the nulliparity of the host mosquito. This is certainly true of *Arrenurus* spp. which parasitize female mosquitoes, since they attach on mosquito emergence and usually detach at first oviposition, the act of egg-laying by the host initiating detachment of the larval mites (McCrae, 1976). Thus *Arrenurus* mites are rarely found on parous mosquitoes and their presence can be taken as an indicator of nulliparity (Corbet, 1960, 1963, 1970; Jalil and Mitchell, 1972; Wharton, 1959). The present results showed that *Arrenurus* mite infection could be used as a convenient marker of host nulliparity in the Sri Lankan *Culex* species studied.

Parasitism by water mites has been shown to reduce longevity in *An. quadrimaculatus* Say and *An. crucians* Wiedemann in the laboratory (Lanciani, 1986; Lanciani and Boyt, 1977). There is evidence of a similar phenomenon in field population of male and female *An. crucians* (Lanciani, 1979, 1987; Lanciani and Boyett, 1980). These studies indicate that mosquitoes with large parasite loads die sooner than others. The lethal effect may be partially due to an upset water balance (Smith and McIver, 1984). Water mite-induced reduction in host egg production has been reported in *Anopheles crucians* (Lanciani and Boyt, 1977) and *Coquillettidia perturbans* (Smith and McIver, 1984), and evidence of reduced flight

capability in *Mansonia uniformis*, *Ma. metallica* and *Cq. perturbans* (Gillies and Wilkes, 1972; Smith, 1988). Similar studies on these important aspects of the mite-mosquito relationship have not been carried out on the *Culex* species studied here, or on other Sri Lankan mosquitoes. However the present results show that with a low mite load on individual mosquitoes (mean $\pm$ S.D. = 1.45 ± 1.01) and a low level of prevalence (2.66% of the sample), mite parasitism would not be expected to be a significant cause of early adult mortality, reduced fecundity or flight activity in the *Culex* species investigated in the Kandy area and would thus have little impact on the dynamics of pathogen transmission by these mosquitoes.

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